*Seed legislation and policy*

## UPOV: International Union for the Protection of New Varieties of Plants

An intergovernmental organization with the scope of promoting an effective system of plant variety protection, aiming at encouraging the development of new varieties of plants, for the benefit of society

It was established by the International Convention for the Protection of New Varieties of Plants. The Convention was adopted in Paris in 1961 with several revisions thereafter

The objective of the Convention is the protection of new varieties of plants by an intellectual property right

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## UPOV: International Union for the Protection of New Varieties of Plants

- Over 50 countries are member of UPOV including most countries in Europe and North and South America
- Still countries are entering UPOV
- UPOV develops descriptive lists for many crops

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## General information

Registration in national listings and protection of a new plant variety requires that the variety is distinct (D) from any other variety ("variety of common knowledge") and that it is sufficiently uniform (U) and stable (S)

UPOV has developed "Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability," for many species or other variety groupings

A "DUS Test," is based mainly on growing tests, carried out by the authority competent for granting plant breeders' rights or by separate institutions (ie. public research institutes)

The test generates a description of the variety, using its relevant characteristics (e.g. plant height, leaf shape, time of flowering)



## General information (cont)

Individual Test Guidelines are prepared by the appropriate Technical Working Party (government appointed experts, invited experts from third parties and observer organizations – opinion of breeders and related industries also taken into account), and submitted for approval by the Technical Committee

A number of general principles apply across all individual Test Guidelines. These principles are the only resort for the DUS examiner when specific recommendations are not appropriate for a particular set of conditions or in the absence of guidelines for a species or variety grouping under concern

## Design of the DUS Tests

The design of the growing trial or other tests, with regard to aspects such as the number of growing cycles, layout of the trial, number of plants to be examined and method of observation, is largely determined by the nature of the variety to be examined

Guidance on design is a key function of the various Test Guidelines

## Characteristics as the Basis for Examination of DUS

For any variety to be capable of protection it must first be clearly defined by its characteristics

A variety is a plant grouping that can be "defined by the expression of the characteristics resulting from a given genotype or combination of genotypes and can be distinguished from any other plant grouping by the expression of at least one of the said characteristics

In addition to their use in defining a variety, characteristics are the basis for examining distinctness, uniformity and stability

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## The meaning of DUS

### Distinctness

Distinctness is established by a variety being “clearly distinguishable by one or more important characteristics,” from any other variety of common knowledge

### Uniformity

Uniformity is assessed on the basis of a variety being sufficiently uniform in its relevant characteristics, subject to the variation that may be expected from its particular nature

### Stability

Relevant characteristics must remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle

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# Requirements of Material for a DUS test

## Representativeness

The material to be submitted for DUS examination should be representative of the candidate variety (ie. in the case of hybrid and synthetic varieties the material should include the final stage in the cycle of propagation).

## General health

The plant material should be visibly healthy, vigorous, free of pests or diseases and (when seed) have sufficient germination for a satisfactory examination.

## Factors affecting the expression of the characteristics of a variety

The tests should be free of factors (not intended for DUS assessment) affecting the expression of the characteristics, otherwise they should influence to the same extent the entire set of varieties examined

# Characteristics used for DUS testing

## Selection of characteristics

The expression of a characteristic should:

- result from a given genotype or combination of genotypes
- be sufficiently consistent and repeatable in a particular environment
- exhibit sufficient variation between varieties to be able to establish distinctness
- be capable of precise definition and recognition
- allow uniformity requirements to be fulfilled
- allow stability requirements to be fulfilled (ie. producing consistent and repeatable results after repeated propagation)

Commercial value of the characteristic is not a requirement

## Characteristics used for DUS testing (cont)

Types of characteristics

Mainly morphological characteristics are used for the description of the variety. Whole plants or any part of a plant can be assessed and used for distinguishing between varieties

- Morphological: shape, colour, hairiness, waxiness, dimensions, size, number
- Non-morphological: maturity, disease or pest resistance, ploidy level, chemical constituent
- Novel types: biochemical methods, DNA profiling, computerized Image Analysis

Provided that the combination is biologically meaningful, characteristics separately assessed may subsequently be combined and examined for DUS.

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## Characteristics used for DUS testing (cont)

Categories of expression

- Qualitative: are expressed as discrete states. The number of discrete states is not limited and are described by a continuous number system from 1 up to however many states are to be allocated. Absence of a feature is shown as 1 and presence as 9. These characteristics are least influenced by the environment

- Quantitative: show a continuous variation from one extreme to another and usually are more influenced by the environment. The range of expression is divided into a number of states evenly distributed across the scale. A 1 to 9 scale is used with 5 being the mid-point value

- Pseudo-qualitative: the range of expression is at least partly continuous but varies in more than one dimensions and cannot be adequately described by just defining two ends of a linear range

## Examining Distinctness

A. Requirements

A variety must be clearly distinguishable from any other variety whose existence is a matter of "common knowledge". The latter is not restricted to national or geographical borders.

A variety is considered as common knowledge if:

- propagated or harvested material of the variety is commercialized or a detailed description is published
- filed for breeder's rights or entered in registration trials in any country, which is deemed to render that variety a matter of common knowledge from the date of the application
- living plant material exists in publicly accessible plant collections

## Examining Distinctness (cont)

B. Comparison of varieties

- Usually growing trials in relation to all (not always) varieties of common knowledge
- In addition, supplementary procedures may be developed to avoid the need for a systematic individual comparisons. These include:
  - publication of description and invitation for comments or exchange of technical information between members
  - comparison of well documented descriptions without growing trials
  - information requested from the breeder through a technical questionnaire
- A variety may be considered as clearly distinguishable if the difference in characteristics is a) consistent and b) clear
  - assessment of consistency might require more than one growing seasons
  - a clear difference depends on the type of expression of the characteristic

## Examining Distinctness (cont)

C. Interpretation of observations

The way observations of the characteristics are handled and interpreted depends on how they are obtained, their type of expression and the propagation feature of the variety

Visually assessed characteristics

- Without statistics
  - difference in the states of at least one qualitative characteristic
  - difference of two notes for a quantitative characteristic
- With statistics
  - usually non-parametric methods
  - direct pairwise comparisons most reliable

## Examining Distinctness (cont)

C. Interpretation of observations

Measured characteristics

- Self-pollinated & Vegetatively propagated varieties
  - a difference of at least a LSD at a specified *P* level with the same sign over an appropriate period
- Cross-pollinated varieties
  - COYD analysis: the size of the differences should be sufficiently consistent over the years taken into account the between year variation
  - refined COYD analysis: adjustment when environmental conditions cause a significant change in the difference between variety means in a year
  - non-parametric methods

## Examining Uniformity

A. Requirements

A variety is uniform if, "subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics"

Relevant characteristics of a variety include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety

The level of uniformity required for a variety depends on the particular features of its propagation

## Examining Uniformity (cont)

B. Methodology used

In case of little within variety variation, as in truly or mainly self-pollinated and vegetatively propagated varieties, the number of "off-type" plants is evaluated

When the within variety variation is larger, as in cross-pollinated varieties, uniformity is assessed on the basis of the overall range of variation, observed across all the individual plants, with regard to comparable varieties



## Examining Uniformity (cont)

Self -Pollinated and Vegetatively Propagated Varieties

- A plant is to be considered an off-type if it can be clearly distinguished from the variety in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness and the same standards are considered as when comparing a candidate to other varieties
- The acceptable number of “off-types” in a sample tested is based on statistical considerations. TGs recommend the maximum number for a given sample size
- A higher tolerance of “off-types” is accepted in mainly than in truly self-pollinated and vegetatively propagated varieties

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## Examining Uniformity (cont)

Cross-Pollinated Varieties

Relative tolerance limits, for the range of variation, are set by comparison with comparable varieties, or types, already known. This means that the candidate variety should not be significantly less uniform than the comparable varieties

- For characteristics that are either recorded by visual observation of single plants or measured (quantitative characteristics), the acceptable level of variation for the variety should not significantly exceed the level of variation found in comparable varieties already known
- UPOV has proposed several statistical methods for dealing with uniformity in measured quantitative characteristics

## Examining Uniformity (cont)

Hybrid Varieties

The assessment of uniformity in hybrid varieties depends on the type of hybrid

- Single- Cross Hybrids from Inbred Parent Lines: are treated as mainly self-pollinated varieties with an additional tolerance for the self-pollinated inbred parents. The percentage of "off-types" allowed depends on the species and the method of propagation. TGs might provide maximum numbers

- Single- Cross Hybrids not exclusively from Inbred Parents
  For hybrid varieties resulting from at least one cross-pollinated parent, relative tolerance limits should be used, and they should be treated as cross-pollinated or synthetic varieties as long as no other proof is given.



## Examining Uniformity (cont)

Hybrid Varieties

- Multiple-Cross Hybrid Varieties

– In these cases (e.g. three-way crosses or double crosses), a segregation of certain characteristics is acceptable if it is compatible with the method of propagation of the variety. Unless the heredity of a clear-cut segregating characteristic is known, it is treated in the same way as other characteristics in cross-pollinated varieties, i.e. relative tolerance limits, for the range of variation, are set by comparison with comparable varieties, or types, already known

– For setting a tolerance for the occurrence of self-pollinated parent plants, the same considerations apply as for a single-cross hybrid variety

## Examining Stability

A. Requirements

A variety is considered stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle

Relevant characteristics of a variety include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety or not.

## Examining Stability (cont)

B. Methodology used

When a variety has been shown to be uniform, it can also be considered to be stable. Therefore, in general, no separate tests are performed.

If needed however, stability may be tested, either by growing a further generation, or by comparing with the previous material supplied

The stability of a hybrid variety may, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity and stability of its parent lines.

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## DUS testing in the absence of TGs

- Cooperation with other UPOV members that might have experience or have developed National TGs, harmonization of procedures and report to UPOV
- Development of TGs for new crops
  - New guidelines should follow the general principles aforementioned and respecting the guidances contained in TGP/7 (Development of TGs)
  - The testing procedure should be documented, in accordance with the requirements of Test Guidelines, to the extent that experience and information permit.
  - UPOV then should be informed according to TGP/5, (Experience and Cooperation in DUS Testing), so that the information can then be passed on to all members of the Union and consideration can be given to the development of Test Guidelines.

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## Variety list contains:

- Importance of the variety
- Origin of the variety
  - Breeder/representative
  - Parent combination of the cross
- Variety description
  - Morphoplogical descriptions
  - Agricultural performance
  - Resistances and level of resistance
- Crop information
  - Comparative info
  - Cultivation info

*Variety identification*

## Choice of method

Depends on the specific identification requirements

Various concepts of variety identification:

- Identification *in sensu stricto*
- Verification of identity
- Distinctness
- Assessment of purity
- Description

In addition different crops and varieties offer varying possibilities for analysis

*Variety identification*

## Classical taxonomic approach

Is based on the observation and recording of a range of morphological characters. Forms the basis of most current DUS testing procedures.

- Can be expensive and time consuming
- Requires highly skilled and trained personnel
- Expression of characteristics is often altered be environmental factors
- In some species the number of characteristics is limited or not sufficient for discrimination between an increased number of cvs

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*Variety identification*

## Novel methods

Aim to reduce or eliminate the environmental influence, thus enabling the genotype to be observed more directly

- Computerised Image Analysis systems
- Biochemical methods
- DNA profiling techniques

# Seed Production & Quality Management

*Variety identification*

## Novel methods

A. Computerised Image Analysis systems

Used to capture and process morphological information

Provides an objective, quantitative and automated method for the routine measurement of characteristics. Often used for combined characteristics

# Seed Production & Quality Management

*Variety identification*

## A. Computerized Image Analysis

| | |
|---|---|
| 1. Image Capture | Sample |
| Image digitisation | A to D Conversion |
| 2. Image processing | Noise reduction / Image enhancement |
| | Improved image |
| 3. Image analysis | Object detection<br>Information extraction<br>Feature extraction |
| 4. Pattern recognition<br>Statistical analysis | Discriminant analysis<br>Classification |
| Decision making | Interpretation |

# Seed Production & Quality Management

*Variety identification*

## A. Computerized Image Analysis systems

- Potential to measure new characters
- Speed
- Electronic storage & transmission of data
- "Non-destructive" (ie. Photographs, negatives etc can be analyzed as living biological specimens)

Given the improvement in imaging technology and software, and the computer processing power further applications of Image Analysis can be anticipated in the future

*Variety identification*

## B. Biochemical methods

Used to analyze components of plants, primarily proteins and enzymes

Useful compounds are categorized into two main groups:

- Secondary compounds (ie. anthocyanins and other flavonoids, fatty acids)
- Molecular markers (proteins, DNA markers)

# Seed Production & Quality Management

*Variety identification*

## Biochemical methods - Secondary compounds

Examples of tests for cultivar identification

| Type of test | Species |
|---|---|
| Phenol | Rice, oats, barley, wheats, ryegrass, *Poa pratensis* |
| Fluorescence +/- chemical treatment | Ryegrass (roots), oats (seed) peas (seed), soybean (roots), |
| Chromosome counting | Sugarbeet, ryegrass spp., clovers |
| NaOH / KOH | Wheats, sorghum, rice, red rice |
| Vanillin | Beans, barley |
| Anthocyanins (GLC-HPLC) | Flowering and ornamental species |

# Seed Production & Quality Management

*Variety identification*

## Biochemical methods - Molecular markers

A. Proteins & Isozymes

- Seed storage proteins: polymorphic proteins encoded at multiple loci. Mostly used in self-pollinated and vegetatively propagated species
- Isozymes: polymorphic proteins derived from a single locus. Mostly used in cross-pollinated species

The analysis of proteins for cultivar identification is routinely based on various types of gel electrophoresis that are easy to perform and do not require sophisticated facilities

Several standard reference methods have been adopted by ISTA

# Seed Production & Quality Management

*Variety identification*

## Biochemical methods - Molecular markers

### A. Proteins & Isozymes

Self-pollinated & Vegetatively propagated crops

- Storage proteins are extracted from a single seed of each variety
- Proteins are separated with the use of PAGE, SDS-PAGE or IFE
- The presence or absence of a particular band at specific places on the gel forms the criterion to differentiate the cultivars
- Examples:
  - Gliadins and glutenin proteins in wheat
  - Hordeins in barley
  - Legumins in legume
  - Esterases in potato

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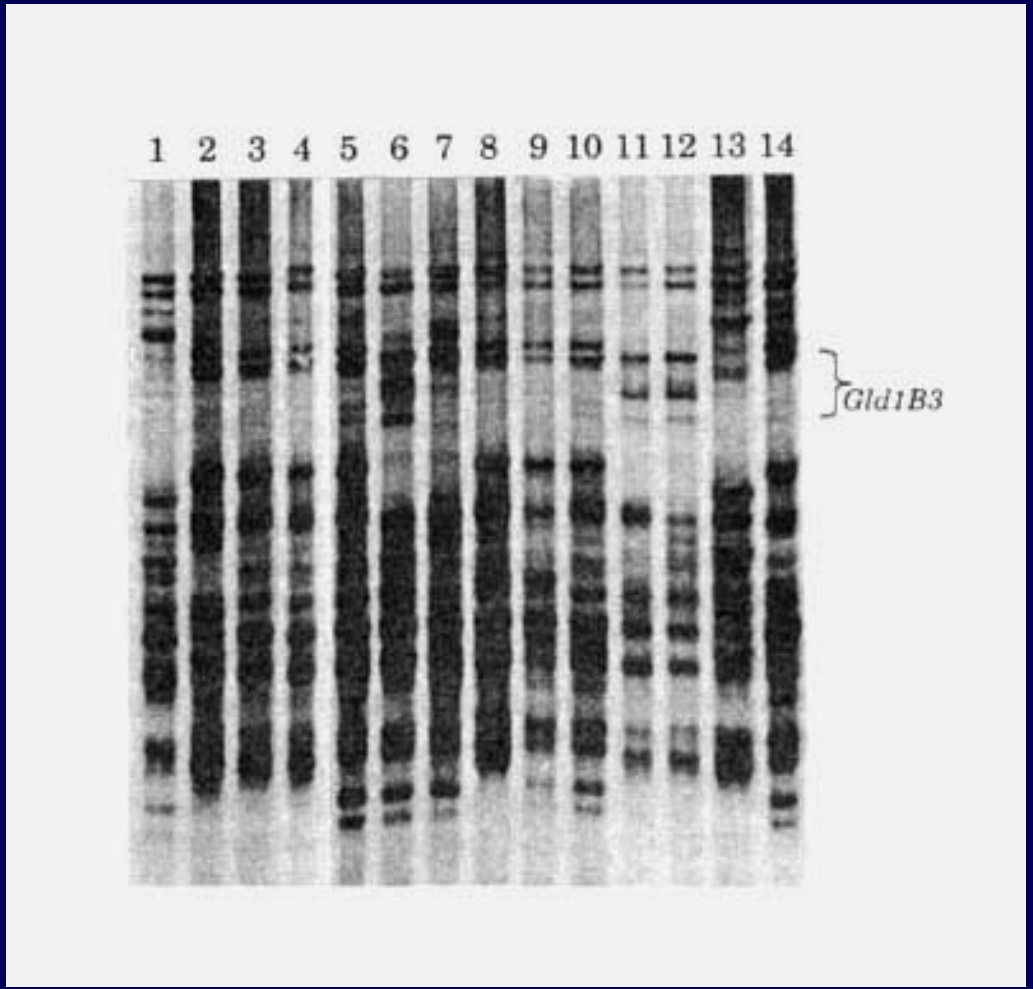
## Biochemical methods - Molecular markers

A. Proteins & Isozymes




*Pattern of gliadin variation in a group of Chinese wheat cultivars*

*Variety identification*

## Biochemical methods - Molecular markers

### A. Proteins & Isozymes

Cross-pollinated crops

To cope with the genetic variation present two approaches exist:

- Analysis of a bulked extract of seeds or plants to obtain an overall cultivar protein profile
- Analysis of isozymes from individual seeds or plants and determination with statistical evaluation of the variability within and between cultivars. Thus, the cultivars are differentiated on the basis of differences in the frequency of occurrence of specific alleles. Combination of data from more isozymes enhances the discriminative power
- Examples: ryegrass, rye, sugarbeet, vegetables, fruits, etc

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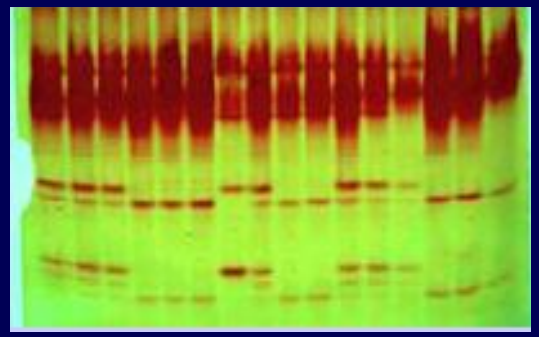
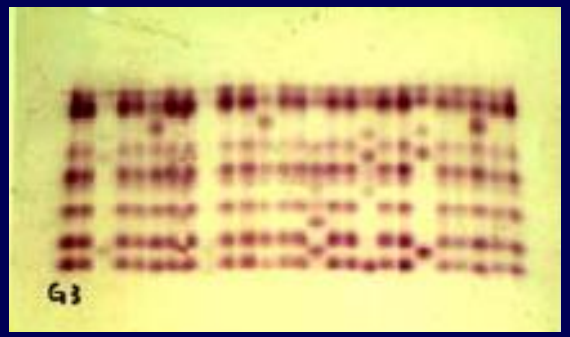
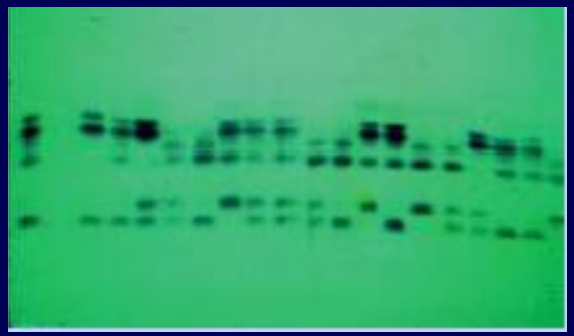
## Isozyme analysis



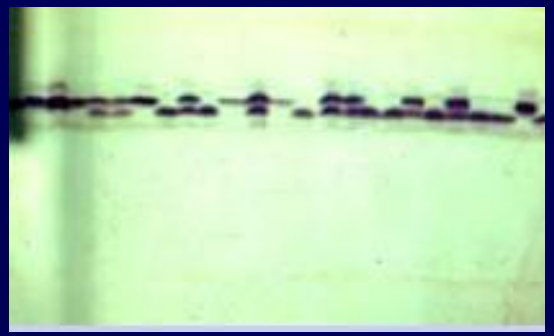
APS (acid phosphatase)
*Brassica oleracea*




PGM (phosphoglucomutase)
*B. oleracea, B. rapa*



PGM (phosphoglucomutase)
*B. oleracea*



LAP (leucin aminopeptidase)
*B. oleracea*

*Variety identification*

## C. DNA profiling techniques – DNA markers

The use of polymorphic markers at the DNA level is more advantageous due to their abundance and independence from environmental conditions and developmental stage of the plant

- Hybridization or probe-based DNA markers (ie. RFLPs)
- DNA markers based on amplification technologies (ie. RAPDs, SSRs)
- DNA markers based on combination of the above (ie. AFLPs)

*Variety identification*

## C. DNA profiling techniques

Probe-based technologies

- DNA samples from contrasting individuals or populations are digested with a restriction enzyme
- DNA is separated by gel electrophoresis
- The DNA is then subjected to Southern blotting
- The DNA probe used must span the restriction site

*Variety identification*

## C. DNA profiling techniques

Probe-based technologies

- Restriction fragment length polymorphisms (RFLPs): utilizes single-, low- or multi-copy genomic of cDNA clones as probes following restriction of the target DNA
- Can be a very discriminating means of profiling
- Requires large amounts of good quality DNA and use of radioactivity

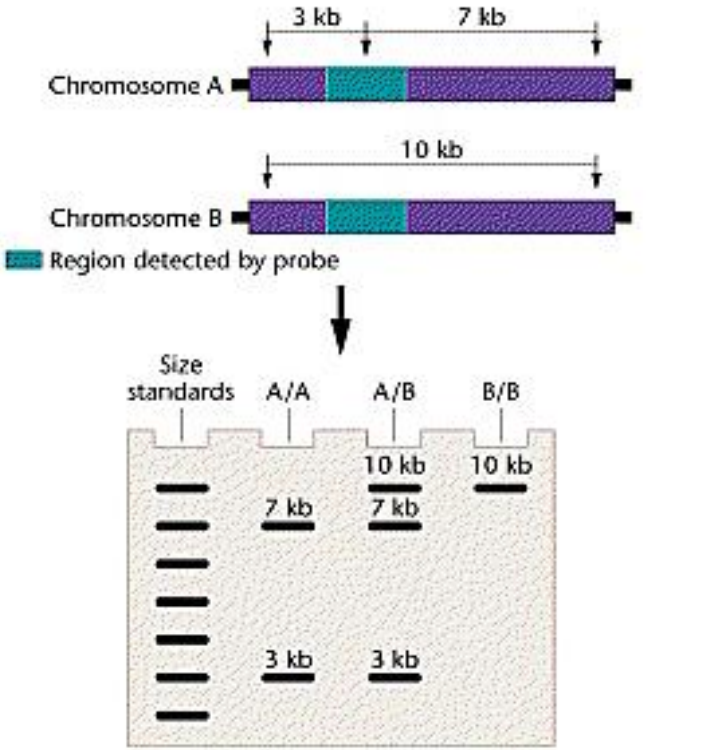
*Variety identification*

## C. DNA profiling techniques

Probe-based technologies

RFLPs

- Co-dominant
- Requires:
  - single copy DNA probe
  - restriction enzyme
  - Southern blotting
  - DNA polymorphism




| Genotypes | Fragment sizes |
|---|---|
| Homozygous for chromosome A (A/A) | 3 kb, 7 kb |
| Heterozygous (A/B) | 3 kb, 7 kb, 10 kb |
| Homozygous for chromosome B (B/B) | 10 kb |

*Variety identification*

## C. DNA profiling techniques

Amplification technologies

- Random amplified polymorphic DNA (RAPD) is most widely used: utilizes the polymorphisms that result by the use of different primers
- RAPD require no prior knowledge of the species DNA sequence, primers are commercially available
- PCR-based methods are quicker than probe-based techniques, require less target DNA, avoid use of radioactivity and can be automated
- Suffer from lack of robustness, problems with interpretation, and reproducibility of the results

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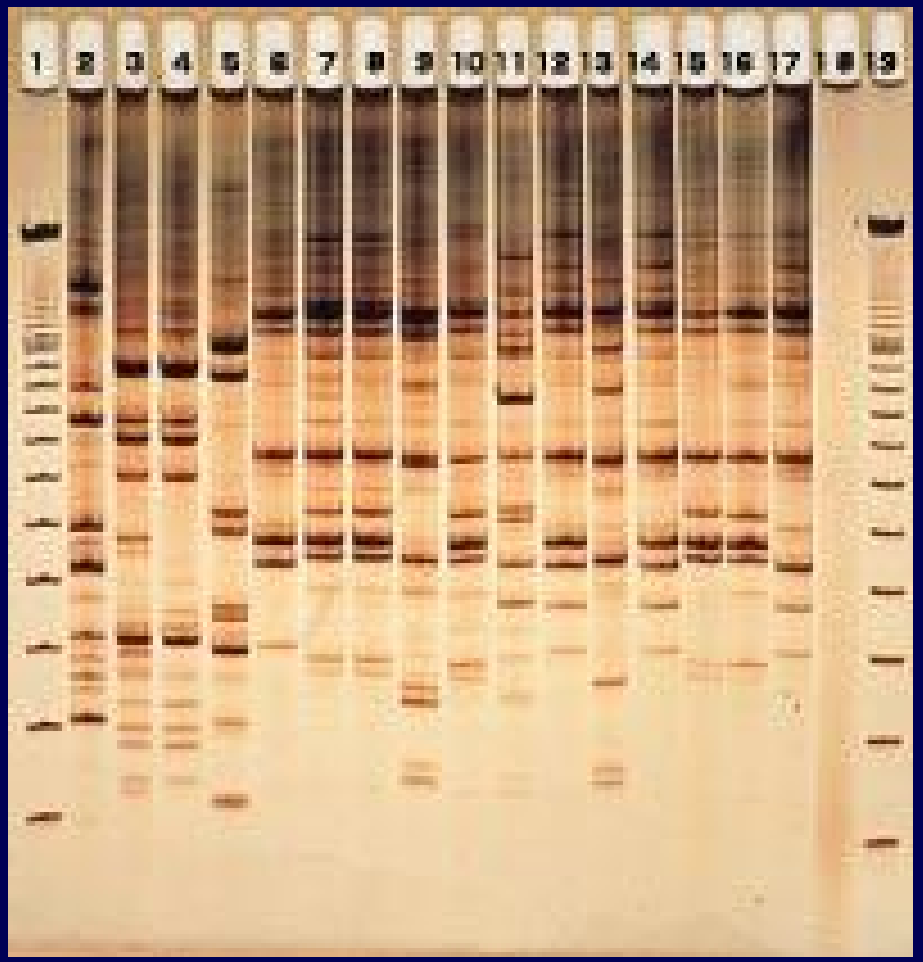
## C. DNA profiling techniques

Amplification technologies

RAPDs

- Randomly amplified polymorphic DNA
- Based on a 10 bp single arbitrary primer
- Cheap, easy
- Insufficient reproducible



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## C. DNA profiling techniques

Amplification technologies

Simple Sequence Repeats (SSR) / microsatellite

- Isolation of DNA fragments (vectors) containing a simple sequence repeat (microsatellite), *e.g.* [GATA], [CGA], [GC]
- Sequencing regions flanking the SSR
- Designing primers for border sequences
- Testing in population for duplications and SSR polymorphism

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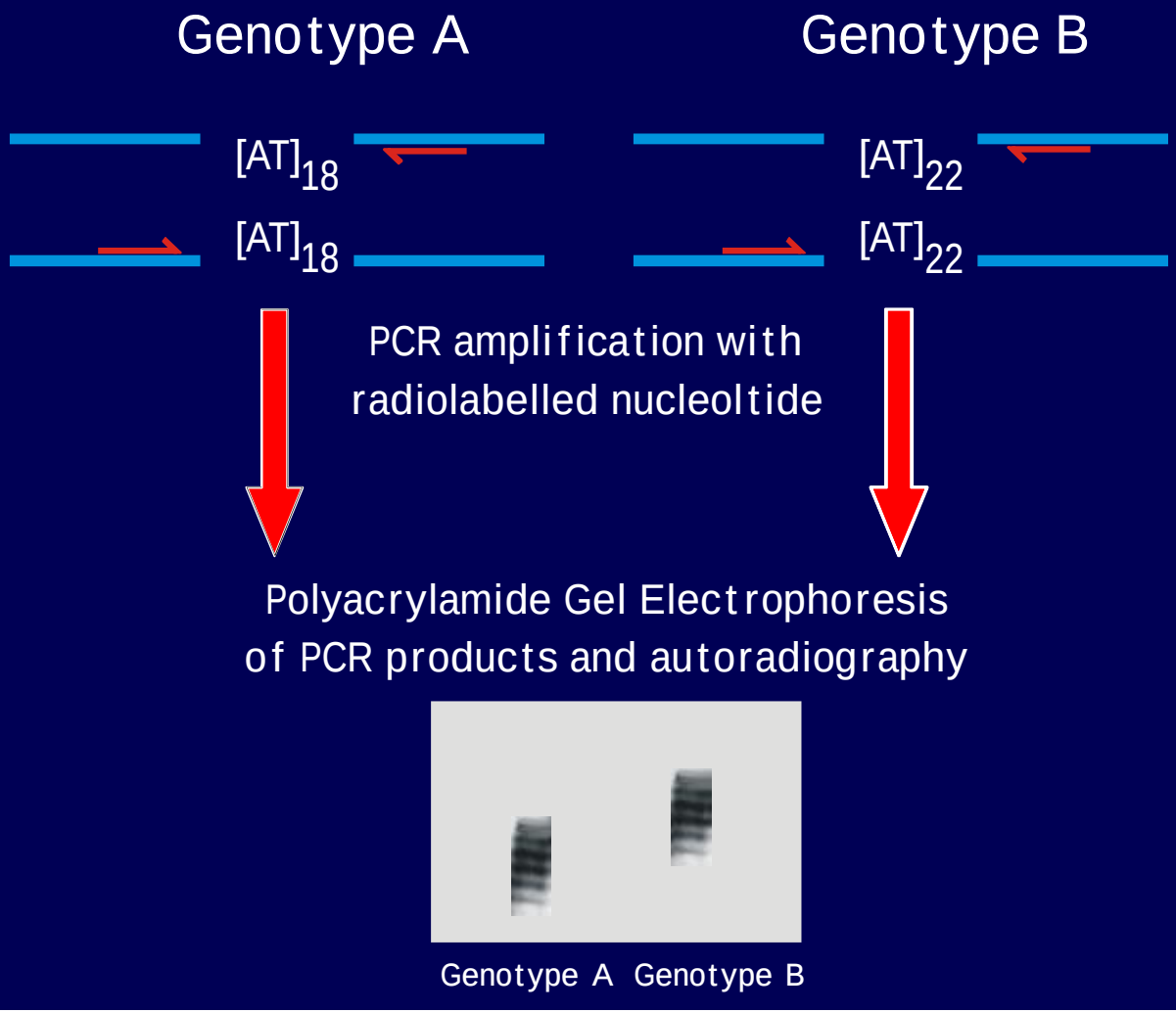
SSRs



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## C. DNA profiling techniques

Combination of hybridization and amplification technologies

Amplified Fragment Length Polymorphism (AFLPs)

- Genomic DNA double digests with a frequent cutter (ie. 4-cutter) and a rare cutter (ie. 6-cutter)
- Ligate adapters to the restriction sites
- Primers complementary to adapters with selective nucleotides at 3' ends and perform PCR amplification
- Separate DNA fragments on high-resolution gels
- After detection, screen for band polymorphisms

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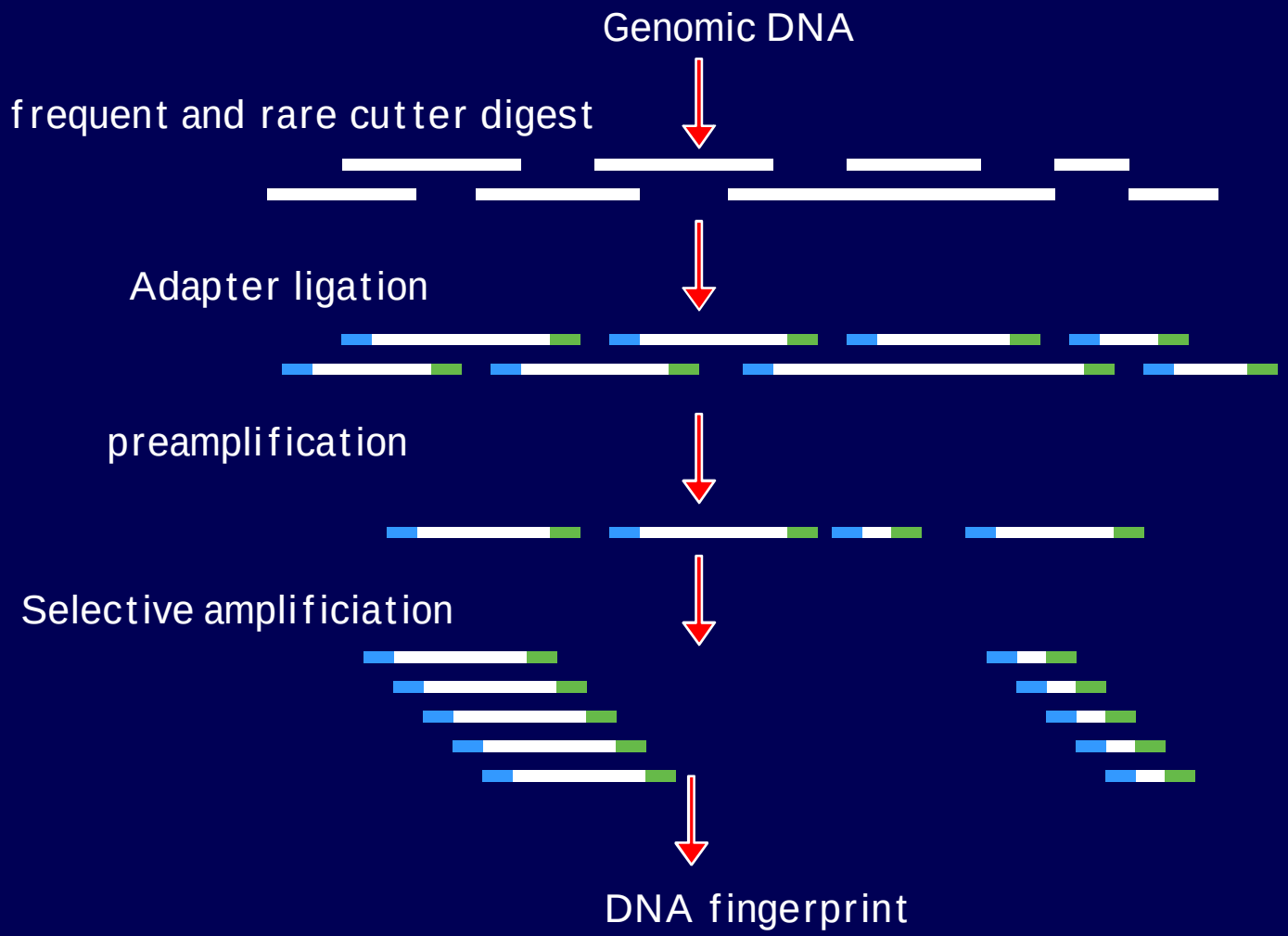
## AFLPs



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## AFLPs